AN EXPERIMENT ON THE DIFFUSION OF SALMONELLA CELL SUSPENSIONS THROUGH FILTER PAPER STRIPS.

Report by Tetsuo Iino (January 12, 1958)

The report deals with a preliminary experiment to separate the mixtures of two different serotypes or of motile and non-motile cells by differential diffusion on filter paper strips.

Materials and Methods.

In the experiment 1, the following two stable monophasic strains were used: a Gal$^-$ Fla$^+$ b-type strain obtained from S. abony SW803 -x S. para B SW666, and a Gal$^+$ Fla$^+$ i-type strain, SW1180. Each of them was grown overnight in penassay broth and their Gal and antigen characters were confirmed on the samples which were streaked onto EMB-gal plates before the cultures were mixed.

A 8mm wide filter paper roll (for paper chromatography) was cut to 160mm long pieces, and each of them was hung in a test tube (18mm x155mm) with metal cap, as shown in figure 1. Five such test tubes were prepared. In the test tubes 2 and 3, 0.2 ml of 1/100 dilution of anti-b and anti-i serums were dropped correspondingly at the bottom of the tubes(the serum does not touch the filter papers). In the tubes 4 and 5, 0.02 ml of 1/10 dilution of anti-b and anti-i serum solutions were correspondingly dropped on the filter paper strips at 5 cm from the lower end. The serum solution diffused 2 cm up and down from the point spotted. The filter papers were stayed until they were dried.

0.5 ml of the each test cultures were mixed in 10 ml of water, and 2 ml of them was dropped at the bottom of each tubes (ca 0.5 mm of the lower ends of the filter papers were immersed in the cell suspensions). They were kept at room temperature, and let the cell suspensions to diffuse through the filter papers. After one and a half hours (the water has reached at the upper end of the filter papers), filter papers were taken out from the tubes, cut in two and printed on EMB-gal plates. Each print

was devided into five sections and was streaked as shown in figure 2. The proportion of two types of cells along the filter paper strips was scored from the ratio of Gal^+ and Gal^- colonies grown from each sections of the EMB-gal plates.

The same experiment was repeated with the mixture of a Gal^+ Fla^- strain, SW1157, and a Gal^-Fla^+ i-type strain obtained from S. typhimurium TM2 -x S. paratyphi B SW666. Anti-b test tubes were excluded from this experiment.

Results and Conclusions.

The results were summarized in table 1. In the first experiment, the proportion of i-type cells decreases at the upper part of the papers in every tubes. That is, the b-type cells diffuse faster than the i-type cells. The effect of antiserum could not be demonstrated. Whether the difference of the diffusion between the two different serotypes relates to the negative charge of the cells or their motility is not clear. The experiment 2 shows that Fla^+ i-type cells diffuse faster than Fla^- cells. Anti-i serums did not affect the result. The method is, therefore, not useful immediately for the screening of non-motile cells . It is suggested to use the higher concentration of antiserums for the farther experiments.

Table 1

The distribution of two cell types along the filter papers.

SECTION ON A FILTER PAPER	Number of Gal^+ and Gal^- cells on each sections (%)															
	+	-	+	-	+	-	+	-	+	-	+	-	+	-	+	-
upper end																
5	1 (25)	3	0	0	0	0	0	0	0	0	0	1	0	0	0	0
4	4 (24)	13	0	1	0	6	0	0	2 (11)	16	0	6	0	0	0	2
3	54 (48)	58	18 (28)	46	20 (47)	23	17 (47)	19	40 (35)	73	4 (4.3)	88	8 (15)	46	9 (18)	42
2	55 (70)	24	59 (58)	42	26 (37)	45	45 (46)	53	17 (44)	22	14 (34)	27	11 (12)	82	30 (38)	50
1 lower end	72 (63)	42	32 (60)	21	77 (71)	32	47 (57)	36	73 (54)	62	30 (31)	68	17 (19)	74	28 (33)	57
0	48 (51)	45									83 (38)	134				
Tube no.	1		2		3		4		5		6		7		8	
Antiserum	/		anti-b in susp.		anti-i in susp.		anti-b on filt.		anti-i on filt.		/		anti-i in susp.		anti-i on filt.	
Mixture of	Fla^+ b Gal^- & Fla^+ i Gal^+										Fla^-(i)Gal^+ & Fla^+ i Gal^-					

Fig. 1

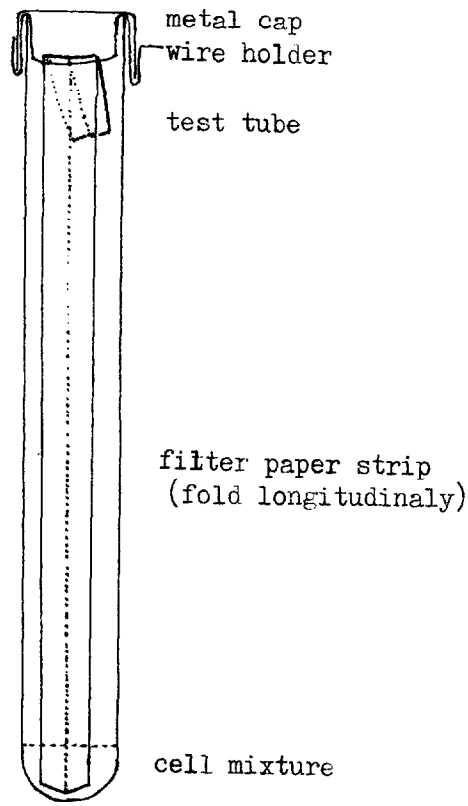
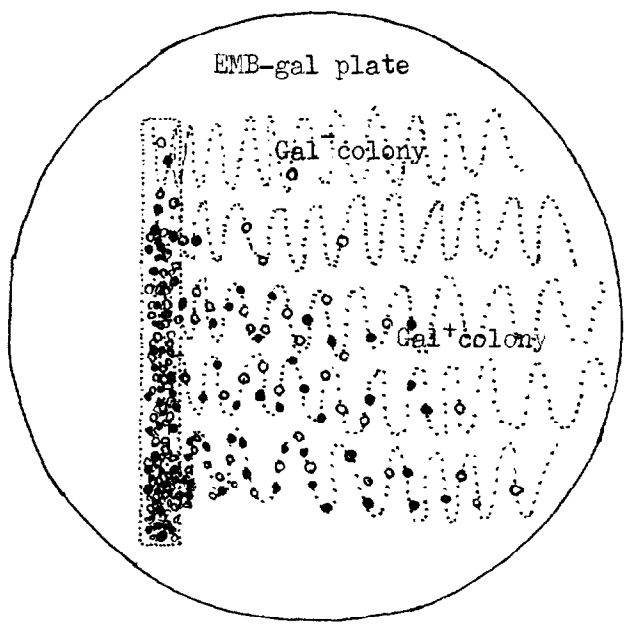


Fig. 2



print of filter
paper strip